Royal Society of Victoria.

# ON BITTER PIT AND SENSITIVITY TO POISONS.

[3RD PAPER.]

BY

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(*Read 11th September*, 1913).

Reprinted from Proc. Roy. Soc. Victoria, Vol. XXVI. (New Series), Pt. II.

ISSUED MARCH, 1914.

*Ford & Son, Printers, Carlton, Melbourne.*

# Art. XVII.—*On Bitter Pit and Sensitivity to Poisons.*

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[3rd Paper].

(With Plate XXIII.)

[Read 11th September, 1913].

In pursuance of former papers in which the extreme sensitivity of apples to poisons was described, it was thought desirable to obtain a comparison with some other plant structure also capable of prolonged semi-dormant existence. For this purpose potatoes were selected, which, like apples, became discoloured by oxidase action when dead, but which, unlike apples, are capable of further growth, and can, for instance, form cork across a cut surface, which apples can not do.

In addition Rothera and Greenwood[1] have recently attempted to gain direct evidence in regard to the poisoning theory of Bitter Pit, and with negative results. Their works and methods appeared to demand further experimental investigation.

## Sensitivity of Potatoes to Poisons.

In order to compare the sensitivity of apples and potatoes to poisons, a number of experiments were performed of which the results are given below. The potatoes were prepared by removing small areas of the skin, and then immersing them in the poisonous solution. They were then cut in two, and the depth to which the brown or purple colour was developed beneath the prepared spots was noted after a few hours' exposure to moist air to allow of the complete oxidation of dead tissues. Snowflake potatoes were used throughout.

MERCURIC CHLORIDE FIVE DAYS IN ONE LITRE OF SOLUTION, TEMPERATURE AVERAGING 14°C.

| Strength of Solution | 1 per 1,000. | 1 per 10,000. | 1 per 100,000. | 1 per 1,000,000. | 1 per 10,000,000. |
|---|---|---|---|---|---|
| Depth of spots. | 4–5 mm. | 1 m.m. | Superficial browning only. | No distinct signs of poisoning. | No signs of poisoning. |

1. Chemical investigation in connection with Bitter Pit, 1913.

COPPER SULPHATE. FIVE DAYS IN ONE LITRE OF SOLUTION, TEMPERATURE AVERAGING 15°C.

| Strength of Solution | 1 per 100. | 1 per 1000. | 1 per 10,000. | 1 per 100,000. | 1 per 1,000,000. |
|---|---|---|---|---|---|
| Depth of spots. | 5–10 mm. | 3–5 mm. | 2–3 mm. | Superficial browning only. | No signs of poisoning. |

With stronger solutions of copper sulphate, when the potatoes are first cut open, a bluish tinge can be seen beyond the browned areas, but after exposure to air all this region browns, and it consists wholly of dead cells.

With solutions of lead nitrate, 1 per 10,000 strength, a faint superficial browning appears on the prepared spots after five days, but with more dilute solutions (1 per 50,000), no signs of poisoning can be detected.

With strong solutions (1 per 1000 to 1 per 10), the tissues beneath the prepared spots remain white, and at first seem to show no signs of poisoning. After a day's exposure to air, however, greyish, scabby spots 1-2 mm. deep develop with a 1 per 1000 solution, and this tissue is made up of dead cells.

Potato juice produces a white flocculent precipitate with lead nitrate, and this action seems to retard considerably the penetration of the lead nitrate. An excess of the latter apparently destroys the oxidase ferment responsible for browning, and potato slices immersed in 10 per cent. lead nitrate remain white for an indefinite length of time. A lesser strength of lead nitrate is needed to kill the pulp cells than to destroy the oxidase. Hence if potatoes are placed in a 5 per cent. solution, beneath each prepared spot is an area of white but dead tissue, and then a brown zone, in which the lead is less concentrated, and the oxidase has had time to act after the cells were killed. Just in front of the brown zone, a white line can be seen, where the cells have been killed, but where no oxidation has as yet occurred, and further in still the cells are living and normal. (See Plate xxiii.) To produce browning, therefore, a time interval is necessary between the death of the cell and the destruction of the oxidase.

With similar exposures at a temperature averaging 13-14° C., the potatoes showed themselves insensitive to 1 per 100 solutions of magnesium sulphate, and 1 per 2000 solutions of barium chlorate, while only a faint superficial browning was produced by a 1 in 50 solution of magnesium sulphate, and a 1 in 500 solution of barium chlorate.

Solutions of potassium chlorate showed no signs of any poisonous action up to strengths in which a strong osmotic action was exercised. Thus even after five days at 13° C. in a 5 per cent. solution, and in spite of the fact that the prepared spots were slightly depressed owing to the withdrawal of water, no signs of any poisoning action could be seen beyond a faint superficial browning on some, but not all, of the prepared spots.

To test the effect of an acid, sulphuric acid was selected; prepared potatoes immersed in it, and examined after one day in the air.

SULPHURIC ACID, EXPOSURE FIVE DAYS, TEMPERATURE AVERAGING 12-13°C.

| 1 per 250. | 1 per 1000. | 1 per 10,000. | 1 per 50,000. | 1 per 200,000. |
|---|---|---|---|---|
| Dead tissue 8–10 mm. beneath each prepared spot, but quite white. | Dead tissue 5–6 mm. deep, quite white. | Pits 2 mm. deep, faint brown. | Faint superficial browning. | No signs of poisoning. |

Sulphuric acid is feebly acid to litmus paper up to 1 c.c. in 100,000 of water, but shows no perceptible acidity with 1 in 1,000,000. Hence litmus is slightly more sensitive to acid than potatoes are. Using potatoes with small sprouts, the latter were completely destroyed and rotted in concentrations up to 1 in 10,000, with 1 in 50,000 the tips of the sprouts were slightly affected, but a 1 per 200,000 solution did not produce any more effect than immersal in distilled water.

Using caustic potash, prepared snowflake potatoes were immersed for five days at an average temperature of 13° C. in $\frac{1}{4}$ litre of solution, then cut open along the line of prepared spots, and examined after 2 hours' exposure to air.

CAUSTIC POTASH.

| | |
|---|---|
| 1 gram per 100 c.c. water. | Pits 3-4 mm. dep. |
| 1 gram per 500 c.c. water. | Pits 1-2 mm. dep. |
| 1 gram per 1000 c.c. water. | Pits superficial to 1 mm. deep. |
| 1 gram per 2000 c.c. water. | Faint, superficial browning only. |
| 1 gram per 5000 c.c. water. | No distinct signs of poisoning. |

Snowflake potatoes appeared to be comparatively insensitive to anæsthetics. Thus with an immersal of 3 days at 13-14° C. in a 5 per cent. solution of ether, the tissue was killed to a depth of 3-4 mm. beneath the prepared spots, but only browned on exposure to air, presumably owing to the ether retarding oxidase action. With a 1 per cent. solution the tissue was killed on the surface, and hardly browned at all, while no signs of poisoning were shown with a 0.2 per cent. solution.

*The influence of temperature.* To test the influence of temperature on the sensitivity to poisons, snowflake potatoes were selected, and mercuric chloride used as the poison. After the removal of fragments of the skin around a median line, they were immersed for 3 days in half a litre of solution. After the exposure they were cut in half through this line, and exposed to the air for a couple of hours. At 30° C., even if the liquid is kept well aerated, the immersal cannot be prolonged further, since after the third day the controls in pure water, which previously are unaffected, begin to show signs of asphyxiation, dead, discoloured tissue then appearing, usually first at the centre. In a 1 per 1,000,000 solution at 30° C. bacteria develop rapidly in spite of all precautions, and hence the solution was replaced by fresh sterile solution daily. With the 1 in 10,000 and 1 in 100,000 solution at 30 ° C., most of the pits were pale at the surface, with a curved dark band deeper in, and on exposure to air in most cases a more diffuse and less darkened zone of dead tissue extended a short distance into the pulp beyond this band. With shorter periods of immersal, the poisoning does not extend beyond the dark band.

| | Concentration. | | |
|---|---|---|---|
| Temperature. | 1 per 10,000. | 1 per 100,009. | 1 per 1,000,000. |
| 29–30°C. | Dark pits 3–5 mm. deep, and tissue dead and darkening nearly to centre. | Pits less dark, 1–3 mm. deep, and diffuse zone beyond darkening to a depth of 2-5 m.m | Slight superficial darkening beneath prepared spots to a depth of 1 m.m. |
| 13–14°C. | Pits 1–2 m.m deep, but not very dark. | Slight superficial darkening only. | No signs of poisoning. |
| 0–1°C. | Slight superficial darkening and brown rim to prepared spots. | No signs of poisoning. | No signs of poisoning. |

SENSITIVITY OF APPLE AND POTATO (Poison Limit at 13–15°C.)

| | APPLE. | | POTATO (Snowflake. | | |
|---|---|---|---|---|---|
| | Concentration. | Mol. Eq. | Concentration. | Mol. Eq. | Ratio. |
| $HgCl_2$ 30°C. | 1 per 10,000,000 | 2710 | 1 per 100,000 | 27.1 | 100 |
| | 1 per 100,000,000 | 27100 | 1 per 1,000,000 | 271.0 | 100 |
| 0–1°C. | 1 per 100,000 | 27 | 1 per 10,000 | 2.7 | 10 |
| $CuSO_4$ | 1 per 1,000,000 | 249 | 1 per 100,000 | 24.9 | 10 |
| $Pb2NO_3$ | 1 per 2,500,000 | 827 | 1 per 10,000 | 3.3 | 250 |
| $MgSO_4$ | 1 per 5,000 | 1.2 | 1 per 50 | 0.012 | 100 |
| $BaCl_2$ | 1 per 10,000 | 3.0 | 1 per 500 | 0.15 | 20 |
| $H_2SO_4$ | 1 per 1,000,000 | 53. | 1 per 50,000 | 2.65 | 20 |
| Alkali | 1 per 10,000 | 0.4 | 1 per 2,000 | 0.05 | 8 |
| KCl | 1 per 10,000 | 0.7 | 1 per 20 | 0.0014 | 500 |
| Anaesthetic | 1 per 100,000 | 11.9 | 1 per 100 | 0.0074 | 1000 |

In all cases, therefore, apples are much more sensitive to poisons than potatoes. The cells of the latter are undoubtedly more actively living, as is shown by the power of forming cork across a cut surface, which the pulp cells of the apple are unable to do. The latter are adult cells, with only a thin lining layer of living protoplasm, specially adapted for prolonged existence in a more or less statical condition, during which their equilibrium is very easily upset by the merest trace of poison. A high temperature affects the sensitivity of potatoes to poison in the same way as it does apples.

## Poisoning Theory of Bitter Pit.

Rothera and Greenwood[1] have recently made an attempt to obtain a direct answer to this problem. They found in the first place that starch grains from both pitted and normal apples would dissolve in diastase, but that in some cases starch grains might still be undissolved after 10 days, thus confirming the results obtained by me in 1912[2], which also showed that resistant grains will usually dissolve after treatment with dilute hydrochloric acid, for a reason to be given later. In regard to the cell-wall, misled by McAlpine's statement (1st Report, p. 12), that the brown colour of bitter pit is due to a gummy or mucilaginous substance of a pectic character, which colours the cell-walls brown, Rothera and Greenwood investigated the chemistry of the cell-wall, and could find no pronounced indication of a difference of composition between the cell-walls of healthy and pitted tissue. This is not surprising, since the brown colour is due to the formation of an oxidation product of tannic acid which unites with the protoplasm lying within the cell-wall. In the early stages of bitter pit the cell-wall is colourless and unaltered. Any changes in the cell-wall could only be the result of slow impregnation subsequent to death.

Direct tests of the poisoning theory of bitter pit were performed by adding the insoluble ash of bitter pit, and the portion dissolving in 10 per cent. nitric acid to starch—diastase solution. No poisoning action could be detected as compared with controls. This is hardly surprising. One experiment only was performed with bitter pit material, which had been mixed with sand, triturated and used for the extracton of starch. It had, therefore, already been washed, and was again well washed with water, alcohol and ether. The possibility of poisons being washed out

1. Chemical investigation on Bitter Pit, 1913.
2. Proc. Roy. Soc. of Victoria, vol. xxiv. (n.s.), p. 416.

by this treatment was overlooked. The bitter pit ash obtained by incineration contained over 70 per cent. of added sand, and the possibility of a formation of insoluble silicates (lead silicate, etc.) needs consideration. Finally, 10 per cent. nitric acid is not a general solvent for metals or metallic oxides, and in insoluble form a poisonous metal is comparatively harmless.

In other experiments the pitted tissue was directly tested by leaving it in contact with diastase solutions for a day, then filtering off and testing the diastase with starch solution. The authors state that the action of malt diastase was strongly accelerated after contact with boiled and unboiled bitter pit pulp, and normal pulp, and that with taka diastase, practically no effect was exercised. On repeating these experiments with filtered solutions of malt diastase dissolved in distilled water, I am able to give them emphatic contradiction. Prolonged contact with pounded apple pulp, boiled[1] or unboiled, bitter pit or normal, practically destroys diastase in 1 to 3 days[2], and unboiled pulp, if anything, appears to be more active than boiled.

Even contact for a short time with pounded apple pulp retards or inhibits diastatic action. Thus (A) 20 grams of pounded apple pulp, and (B) 20 grams previously heated to 100° C. for 15 minutes, were added to separate 10 c.c. of 1 per cent. Taka diastase, and at once filtered. In three hours 5 c.c. of each filtrate were added to 10 c.c. of 0.5 per cent. starch, and a control contained 10 c.c. of 0.5 per cent. starch to 5 c.c. of 0.33 per cent. Taka diastase.

The solutions were kept at 35° C., and portions tested at intervals with iodine. In (A) the liquid was brown, and developed a large, brown coagulum, which was shaken up before testing. In (B) the liquid was colourless, and formed a smaller precipitate, later nearly all dissolving.

| | 3 Hours. | 6 Hours. | 16 Hours. | 30 Hours. |
|---|---|---|---|---|
| (A) | Blue. | Blue. | Blue. | Blue. |
| (B) | Blue. | Purplish blue. | Purple. | Purple. |
| Control | Paler blue. | Purple. | Nil. | Nil. |

On testing (A) and (B) the former contained distinctly more tannic acid. Apparently in the heated pulp a good deal of the tannic acid combines with the coagulated proteids of the cell.

1. Out of contact with water.

2. With prolonged exposure a portion of the action appears to be due to the diastase destroying itself when in solution even at low temperatures such as 14 or 15°C. (See Czapek, Biochemie, vol i., p. 345.)

The apparent accelerating action on malt diastase observed by Rothera and Greenwood is the result of an experimental error, and is probably due to the action of the tannic acid of the apple pulp upon the starch solution, and upon the iodine test employed.

On mixing 1 per cent. solutions of gallotannic acid and starch a dense white precipitate is thrown down. With more dilute solutions up to 0.1 per cent. a more bulky and gelatinous precipitate forms. A slight gelatinous precipitate may form even when 0.04 per cent. solution is used. These results are shown even when filtered starch solution is used, but the settling of the coagulum or precipitate is slower. The precipitate dissolves on boiling, and is precipitated on cooling, even in the presence of dilute HCl. On repeatedly washing the coagulum with water all the tannic acid can be removed, and it then gives no appreciable blue with ferric chloride. If strong tannic acid is poured into starch solution, pasty masses of plastic starch form. On boiling in water these break up, but without completely dissolving, especially if they have been for some time in contact with the tannic acid. They dissolve, forming a clear solution on warming with dilute hydrochloric acid, but on cooling a white precipitate of starch is formed. The coagulum is not strongly attacked by diastase. At least in experiments lasting over 1-2 days at 30° C., the precipitated starch was still undissolved by 1 per cent. malt diastase, and gave a strong blue with iodine. After drying in air the plastic masses of precipitated starch became hard and translucent. They did not dissolve even on prolonged boiling with water, but dissolved rapidly on the addition of hydrochloric acid, forming a clear solution, giving blue with iodine. This "insoluble" starch is probably in a different physical condition to the starch grains, and appears to be very resistant to diastase.

An excess of cold 2 per cent. tannic acid even precipitates soluble starch, but the milky liquid becomes clear again at 35° C., and cloudy on cooling, while the dried gummy precipitate dissolves readily in boiling water. Gallic acid is much less active than gallotannic acid in precipitating ordinary starch, and has no precipitating action on soluble starch, even in considerable excess. In addition Heintz[1] has shown that tannic acid interferes with the iodine test for starch. Thus, if a drop of iodine solution is added to a mixture of a 1 per cent. tannic acid and 1 per cent. starch solution, the blue rapidly fades to purple, and then

1. Jahresb. Agrikult.—Chem., 1879, p. 499.

colourless. On adding excess of iodine, the blue colour is permanent. As the precipitated starch is allowed to settle, the liquid above gives colour reactions with iodine very like those occurring during diastatic action, and in the presence of tannic acid, a small amount of starch is easily overlooked. This is probably the explanation of the erroneous statement made by Rothera and Greenwood, in regard to the accelerating action of contact with apple pulp, on malt diastase. If 0.5 per cent. solutions of diastase and tannic acid are mixed and filtered, the filtrate throws down a white coagulum with starch solution, which may still be undissolved, even after 3 days.

The retardation of amylolysis by tannin was first shown by Payen, but the action has been considered to be due to the tannic acid precipitating the diastase. (Czapek, Biochemie, vol. 1, p. 344). The following experiments are given in full, since they show the exact action of the tannic acid. Needless to say, before each sample was taken, the liquid was shaken, so that any precipitated starch was evenly distributed, and the proper amount of iodine was used in each case.

Tannic acid produces a bulky white precipitate, with ordinary papain and pepsina porci, but pure pancreatin and diastase remain clear, or shew only a slight cloudiness. By treatment with alcohol, and washing active diastase can be recovered. Gallic acid does not precipitate any of these ferments appreciably, if clear solutions are used, and the amount of precipitate formed with tannic acid appear to depend upon the amount of contamination with foreign coagulable proteids. The action of the tannic acid is on the starch rather than upon the diastase, and hence gallic acid, which is a feebler starch coagulin, affects diastase action less than tannic acid does.

In the first experiment (A) 5 c.c. of unfiltered 1 per cent. diastase was added to each 5 c.c. of filtered 1 per cent. starch solution, and 5 c.c. of the solution of gallotannic acid to each mixture. In experiment (B) filtered 1 per cent. diastase was used, and the temperature kept between 26° and 28° C. Portions of each solution were tested at regular intervals of time with iodine, the colour sequence as the starch dissolves being blue, violet, purple, brown, yellow, the last being merely due to the iodine, and being given as nil.

TABLE A.

| Tannic Acid Solution added. | Iodine Test After | | |
|---|---|---|---|
| | 3 Hours. | 16 Hours. | 26 Hours. |
| 5 c.c. of 5%* | Strong blue. | Strong blue. | Starch in dense ppt., liquid starchless. |
| 5 c.c. of 1% | Strong blue. | Strong blue. | Nearly all starch pptd. |
| 5 c.c. of 0.2% | Strong blue. | Strong blue. | Strong blue. |
| 5 c.c. of 0.4% | Paler blue. | Paler blue. | Violet. |
| 5 c.c. of 0.008% | Purple. | Purple. | Nil. |
| 5 c.c. of 0.0016% | Purple. | Nil. | |
| 5 c.c. of 0.0010% | Nil, all dissolved. | | |
| 5 c.c. of distilled water | Nil, all dissolved. | Nil. | |
| 5 c.c. of 1% starch and 10 c.c. of 2.5% tannic acid | Strong blue. | Dense residue of starch; liquid, starch free. | |

*Equalling in the total 15 c.c. of solution, a concentration of 1.6% of tannic acid ($\frac{1}{3}$ of the concentration of the 5 c.c. added in each case.

TABLE B.

| Tannic Acid Solution added. | Iodine Test After | | | | |
|---|---|---|---|---|---|
| | 1½ Hours. | 3 Hours. | 6 Hours. | 18 Hours. | 30 Hours. |
| 5 c.c. of 1%* | Blue, and white ppt. | Blue. | Blue. | Blue. | Blue. |
| 5 c.c. of 0.2% | Blue, and gelatinous ppt. | Blue. | Blue. | Blue. | Blue. |
| 5 c c. of 0.04% | Blue. | Blue. | Blue. | Blue. | Blue. |
| 5 c.c. of 0.01% | Blue. | Blue. | Blue. | Blue. | Blue. |
| 5 c.c. of 0.005% | Blue. | Blue. | Pale blue. | Purple. | Nil. |
| 5 c.c. of 0.001% | Paler blue. | Purple. | Nil. | | |
| 5 c.c. distilled water. | Paler blue. | Purple. | Nil. | | |

*Total concentration one-third of this in each case.

Similar results were obtained with Taka diastase, except that it appeared to be a little less sensitive than the samples of malt diastase used. Possibly on this account the retarding action of the 0.02 per cent. solution was not so pronounced, and it required from 0.2 to 0.08 per cent. solutions to practically arrest the diastatic action. At least much undissolved and condensed starch was still present in the latter case after 30 hours. A portion of the difference was due to the higher temperature, and with malt diastase at 35° C., 0.2 to 0.08 per cent. tannic acid was also necessary to prevent the complete solution of the starch in 30 hours.

To each 5 c.c. of 1 per cent. Taka diastase, and 5 c.c. of 1 per cent. solution, 5 c.c. of tannic acid were added, and the mixture

kept at 35° C., and tested at intervals with drops of iodine solution. With the 1 per cent. and the 0.2 per cent. solutions of tannic acid a large coagulum of starch was formed condensing on standing. With 0.04 per cent. a slight coagulum was formed, later nearly dissolving.

| Tannic Acid. | 3 Hours. | 9 Hours. | 20 Hours. | 30 Hours. |
|---|---|---|---|---|
| 5 c.c. of 1 %* | Strong blue. | Strong blue. | Strong blue. | Ppt. smaller but giving strong blue. |
| 5 c.c. of 0.2% | Strong blue. | Strong blue. | Strong blue. | Ppt. smaller but giving strong blue. |
| 5 c.c. of 0.04% | Strong blue. | Fairly strong blue. | Purple. | Pale purple. |
| 5 c.c. of 0.02% | Fairly strong blue. | Fairly strong blue. | Pale purple | Nil. |
| 5 c.c. of 0.008% | Paler blue. | Greenish blue. | Nil. | Nil. |
| 5 c.c. of 0.001% | Greenish blue. | Greenish blue. | Nil. | Nil. |
| 5 c.c. of water | Greenish blue. | Purple. | Nil. | Nil. |

* Total concentration one-third of this in each case.

There are some indications that even after adding 5 c.c. of 0.2 per cent. tannic acid (making a concentration of 0.07 per cent. approximately in the whole liquid), a small amount of the precipitated starch was converted into sugar at 35° C., and there is slightly less precipitation when the solutions are warmed to 38° C. before mixing them if they are mixed at 14° or 15° C. Accordingly in the following experiment the solutions were all warmed to 38° C. before mixing, and kept at that temperature for 24 hours. Each tube contained 5 c.c. of 1 per cent. starch, 5 c.c. of 1 per cent. diastase, and 5 c.c. of tannic acid. The controls were boiled. The tubes were shaken several times during the 24 hours, and then 10 c.c. of the supernatant liquid tested with Fehling's solution, using an excess of sodium hydrate. The brown precipitate dissolves, forming first a green, and then a brown liquid, and tests showed that even in the presence of tannic acid small quantities of sugar can be detected. Tannic acid itself gives a red precipitate[1] with Fehling's solution, if the sodium hydrate is not in excess. If the latter is in excess the tannic acid reduction is separated by a long interval from the sugar reduction, or is practically arrested. The red precipitates were allowed to settle for not more than 10 or 15 minutes, filtered rapidly on tared filter papers, washed, dried and weighed, and the excess weight over the control is given in the third column of the table.

1. According to Sonnenschein, Ding. Polytechn. Journ., Vol. cclvi., p. 555, 1885, 1 gram CuO = 0.4126 gram tannin and 0.4245 gram dextrose.

| Concentration of Tannic Acid in whole solution. | | Supernatant liquid. | Reduction to Fehling's test. | Residue. | Iodine Test. |
|---|---|---|---|---|---|
| 0.33% | Unboiled. | Milky. | Weak. (0.00 gram.) | Small, but condensed. | Strong blue. |
| | Boiled control. | Very milky. | Weak.* | Small, but condensed. | Strong blue. |
| 0.16% | Unboiled. | Clear. | Slightly stronger. (0.01 gram.) | More bulky. | Strong blue. slightly purplish. |
| | Boiled control. | Milky. | Weak. | Small, but condensed. | Strong blue. |
| 0.033% | Unboiled. | Clear. | Distinctly stronger. (0.025 gram.) | Smaller. | Strong, but distinctly purplish blue. |
| | Boiled control. | Milky. | Weak | Still smaller but more condensed. | Strong blue. |

*The diastase used gave a faint reduction with Fehling's test.

Even at optimal temperatures therefore tannic acid distinctly retards diastatic action in a concentration of 0.0003 to 0.003 per cent. (only 1-10th of the starch having dissolved), strongly retards it in concentrations of 0.003 to 0.06 (1-25th dissolved) and practically inhibits it in concentrations of 0.33 to 1 per cent.

Tannic acid may therefore be regarded as a "coagulin," or "anti-diastase." Its inhibitory action appears to be much more pronounced at 15-20° C. than at 35° C. and the average temperature at which the metabolism of the apple takes place lies within the former limits. Now, P. R. Scott found the sap of pitted Wolsely apples contained 0.106, and that of clean apples 0.074 of tannic acid per 100 c.c. of juice. At first sight we might seem to have here an explanation of the non-solution of the starch grains in bitter pit.

The resistance of apple diastase to tannic acid has, however, still to be determined. In addition the diastase of the pulp cells is in their protoplasmic lining, whereas the tannic acid is in the cell-sap within. It is only when the protoplasm is killed that tannic acid penetrates it, and there it comes into contact with the oxidase ferment, and is oxidized to a brown colour. The absence of the latter so long as the cell is living shows that the diastase and the oxidase ferments are not in contact with tannic acid, although the vacuolar membrane separating them may represent a space of less than a thousandth part of a millimetre. Further there is no evidence to show that the percentage of tannic acid is higher prior to bitter pit formation, and an increased percentage of tannic acid often follows as the result of injury or stimulation

to a plant tissue without exercising any additional injurious influence.

Any starch grains, however, which were extruded into the cell-sap would be protected from diastatic action, and hence it was of interest to determine their actual position in bitter pit cells, and in those occasional starch-bearing cells, which so frequently occur in healthy pulp tissue. Sections of bitter pit and healthy pulp were stained with iodine, and examined in a horizontal microscope. After noting the position of the starch grains, the stage was rotated through 180°. It is easy to see in this way that certainly 99 per cent. of the starch is in the protoplasm. Very occasionally a large starch grain can be seen to be lying in the vacuole, and to move when the cells are turned upside down. The same is shown in carefully teased preparations. In the brown starch bearing tissue formed by bruising an apple when it is in the starch stage of development, free starch grains lying in the vacuole are much commoner, but even here by far the greater number of the starch grains always remain in the dead protoplasm. Any starch grains lying in the vacuole after prolonged contact with tannic acid would become more resistant to diastatic action. Probably this is the explanation of the occurrence of occasional resistant grains in the starch of both normal and bitter pit pulp. The fact that treatment with dilute hydrochloric acid makes these grains more readily dissolved by diastase is not necessarily due, as I formerly supposed to be the case, to the removal of a poison inhibiting ferment action, but is possibly due to the acid removing the peculiar insoluble or difficultly soluble condition into which contact with tannic acid throws starch.

### *The Diastase Method of Detecting Poisons.*

That this method of detecting extraneous poisons in bitter pit tissue would be a failure might have been predicted from the foregoing facts. Indeed, since metallic poisons usually combine with the proteids of the cell, a cell which had just received enough to poison it might have little or no available surplus to poison a second cell or a solution of diastase.

Cubes of apple pulp weighing 5 grams were floated for two days on (a) water, (b) 1 per 1,000,000 mercuric chloride: (a) browned on the surface, (b) more deeply, and a duller brown. Each was then pounded up, 10 c.c. of 0.05 per cent. diastase added and filtered after each day. 5 c.c. of each filtrate were added to

10 c.c. of dilute 0.05 per cent. starch solution. In the control all the starch dissolved at 35° C. in 3 hours, but even after 1 day abundance of starch was present both in (a) and (b), and a large coagulum separated out containing nearly all the starch. After two days the starch was still undissolved, but more reducing sugar seemed to be present than in the control. The clear liquid turned pale yellow with NaHO , and contained tannic acid derived from the apple sap, but a good deal of the tannic acid combines with the proteids of the tissue, or is carried down by the precipitated starch.

In a similar test there were added to 10 c.c. of 0.5 per cent. diastase and 10 c.c. of 0.5 per cent. starch (a) 10 c.c. of distilled water, (b) 10 c.c. of boiled filtered apple sap, and (c) 10 c.c. of 1 per 1,000,000 $HgCl_2$. Both (a) and (c) remained clear, and the starch dissolved in $3\frac{1}{2}$ hours, whereas in (b) a white coagulum of starch formed which was not entirely undissolved, even after 3 days at 25° C. The clear supernatant liquid before shaking gave no reaction with iodine, just as though all the starch had been dissolved instead of merely the unprecipitated starch.

Similar experiments with equal volumes of solutions of mercuric chloride, 0.2 per cent. malt diastase, and 0.5 per cent. starch, showed that a 1 per 1,000,000 solution of mercuric chloride exercises no appreciable influence upon the diastase, whereas a 1 per 10,000 solution appeared to stop the diastatic action entirely.

The pulp cells of apples are evidently much more sensitive to mercuric chloride than is diastase or diastatic action. In bitter pit formation, however, the arrest of diastatic action comes first, and the death of the cell follows later. The diastase of apples is either small in amount, or feeble in activity, as compared with malt diastase, and the solution of the starch grains in ripening apples may take not a few hours, but several weeks to complete, so that a feeble diastatic activity might be suppressed or retarded by gradually accumulating traces of poison until the concentration was reached at which the protoplasm was killed. Under the conditions of a laboratory experiment where the tests must be completed in a few hours to a day or so, and comparatively large amounts of ferments used, the use of diastase would only detect a poison in the ash of bitter pit tissue when present in relatively large amount. It would not necessarily detect an amount of poison sufficient to inhibit a feeble diastatic activity, taking normally days or weeks to be completed. It is also conceivable that an amount of poison insufficient to inhibit diastatic action, or to

kill the cell might inhibit the production of diastase, and there is some evidence to show that in certain plants (fungi, etc.), not only poisons, but also food substances may exercise a regulatory or inhibitory action upon the formation of diastase. Dr. White, however, found diastase to be present at least in the early stages of bitter pit. As it is not possible to detect metallic poisons in bitter pit tissue by the diastase method, what is needed is a complete exhaustive percentage analysis of the ash of bitter pit tissue, using large quantities of material, and methods of concentration like those which enable traces of certain metals to be extracted from their ores. No such analyses have as yet been made, and they lie more in the province of the chemist than of the plant physiologist, but the significant fact noted by Mr. P. R. Scott, that the percentage of ash is higher in bitter pit tissue than in normal pulp, merits further investigation.

## Summary.

In all cases potatoes are less sensitive to poisons than apples, the differences in the resistance varying from 1000 times (anaesthetic), to 8 or 10 times (alkali and copper sulphate). Mercuric chloride and copper sulphate are about equally poisonous to potatoes, and sulphuric acid is only slightly less poisonous than lead nitrate, and is some 50 times as poisonous as is alkali.

As in the case of apples, the sensitivity to poisons is much greater at high than at low temperatures. Tannic acid precipitates starch from its solution in water. The precipitate dissolves on boiling, and forms again on cooling, even in the presence of hydrochloric acid. The precipitate can be obtained in gluten-like masses soluble with difficulty, or imperfectly soluble in diastase, and in hot water, but readily soluble in dilute hydrochloric acid on boiling. The occasional resistant starch grains found in apples have possibly been in contact with the tannic acid of the cell-sap. They will dissolve in diastase after warming with dilute hydrochloric acid, and then washing well.

The presence of 0.0003 to 0.003 per cent. of tannic acid distinctly retards diastatic action; 0.003 to 0.06 per cent. strongly retards it, and 0.33 to 1 per cent. practically inhibits it. This effect is shown at 35° C., and is still more pronounced below 20° C. The cell-sap of apples may contain up to as much as 0.1 per cent. tannic acid, and bitter pit tissue appears to contain more than normal pulp. This will protect any starch grains extruded into the cell-

sap from solution, and in cells bruised while in the starch stage, will aid in preventing the starch from dissolving. In the bitter pit cells, however, the starch grains are in the protoplasm, and so long as the latter is living the tannic acid of the cell-sap is not in contact with them, or with diastase. If any of the tannic is present in the protoplasm, it exists in the form of special small vacuoles, and is also not in contact with the starch or diastase. It is probably the difficulty of penetration which makes externally applied tannic acid non-poisonous in dilutions below 1 per 1000. Diastase solution after prolonged contact with pounded apple pulp loses its solvent action.

In the presence of tannic acid a small quantity of starch is easily overlooked by the iodine test, apart from its precipitation, It was possibly in this way that Rothera and Greenwood obtained an apparent acceleration of diastatic action after contact with apple pulp. Their results are therefore of no value so far as the poisoning theory of bitter pit is concerned. A complete exhaustive numerical analysis of the mineral constituents of bitter pit tissue in bulk is urgently needed, paying special attention to metallic elements present in small amount, and this would probably give definite guidance for further investigation. The nature of the combinations in which the mineral constituents occur will also be of importance. Thus approximately 1 per cent. of the ash may consist of oxide of iron. If this were present, either as the chlorides or sulphates, it would be distinctly poisonous.

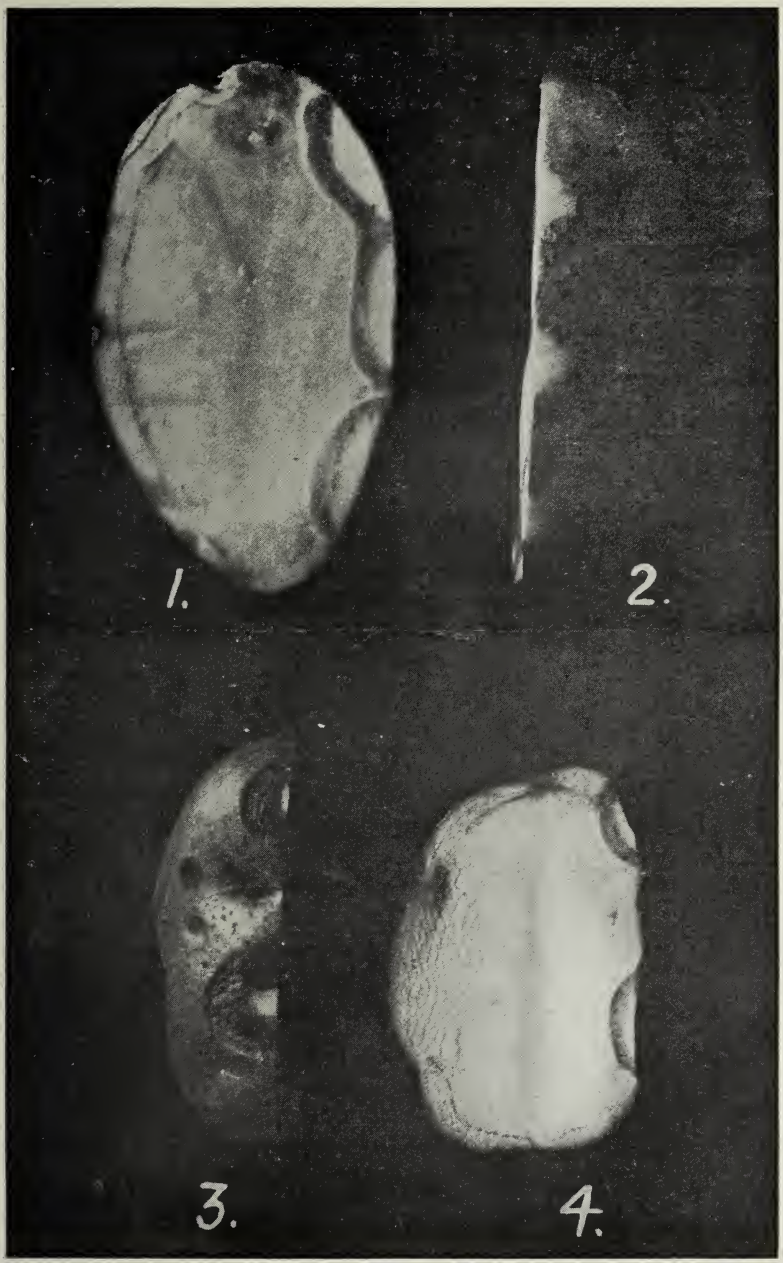
## EXPLANATION OF PLATES XXIII.

Potatoes halved after four days in 5 per cent. lead nitrate.

a and d, face view, b and c, side view of cut half.

Two fragments of skin were removed on c and d, and three larger ones on a and b prior to immersal.

The poison was absorbed from the surface, but the browning is deep-seated.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